Segment III (Method Validation)

Specimen Preparation

Set 1:
- DNA Samples purchased from Coriell Cell Repository with previously characterized genotypes and copy number
- Sample size: n=30
- Ethnic differences in alleles frequencies will be taken account:
  e.g. 5 Caucasian, 5 Japanese, 5 Chinese, 5 African American, 5 South East, and 5 Middle East

Set 2:
- Residual patient genomic DNA samples
- SNVs and CNVs of these samples have been previously characterized by other methods, e.g. Infiniti technology.
- De-identified according to your institutional protocols.

Accuracy (Method Comparison)

Analyze two sample sets (i.e. Coriell DNA extracts and residual patients genomic DNA) by MALDI-TOF/MS.

- 30~40 samples
- 1 non-template control
- Two positive controls (1 duplication control + 1 deletion control)

Compared Genotype and copy number calls generated by MALDI-TOF/MS with those obtained from other technologies (i.e. open array)

Cross-Comparison of Luminex and MALDI-TOF/MS (CYP2D6)

Whole blood samples from venlafaxine treated patients

Luminex

Agena Bioscience MassArray

Sample	LUMINEX	MassArray
1	*5/NEG	*5/NEG
2	*2A/*9	*2A/*9
3	NEG/NEG	NEG/NEG
4	*5/NEG	*5/NEG
5	NEG/NEG	NEG/NEG
6	NEG/NEG	NEG/NEG
7	*2A//*41	*2A/*41
8	*2A/NEG	*35/NEG
9	*4/NEG	*4/NEG
10	*2//*41	*35/*41
11	*2A/*4	*2A/*4
12	*41/NEG	*41/NEG
13	*5//*41	*5/*41
14	*4/*41	*4/*41
15	*5//*41	*5/*41
16	*5/*9	*5/*9
17	DUP *3 /*4	DUP *3/*4

Data generated by Dr. Roberta Melis (ARUP)

Basic Protocols:

Reproducibility & Specimen Type Optimization

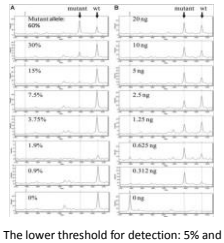
- Within-run:
  - A set of 5 samples with different genotypes will be run in triplicate.
- Between-runs:
  - The same set of samples will be run in triplicates in 5 days.

- Whole blood samples collected in
  - EDTA
  - Heparin
  - Sodium citrate
- N=5-10
- Evaluated genotype calls and confidence scores.

Basic protocol:

Sensitivity

- Two DNA samples at five different concentrations (20, 10, 5, 2.5, and 1.25 ng/µl, respectively) will be tested in duplicates.
- Determine DNA concentrations using fluorimeter (Life technologies) or similar technologies.



- The lower threshold for detection: 5% and 10%.
- Mutant alleles detected: as little as 1 ng DNA per well

Carol Beadling, J Mol Diagn. 2011 Sep; 13(5): 504–513.

Carryover

Basic protocol:

- Evaluate both serial and proximal carryover using 2 high concentration samples (e.g. 20 and 10 ng/ml) followed by and surrounded by no template controls, respectively.
- Evaluate the entire analytical process for possible carryover: extraction, spotting, mass spectrometric analysis.

Summary

- MALDI-TOF /MS is suitable for PGx testing with fast turnaround time, high accuracy, high sensitivity and multiplexing capabilities.
- Implementation of MALDI-TOF/MS PGx assays in clinical laboratory is challenging

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https://www.surveymonkey.com/r/7C55NCJ

Please let us know what training resources you need

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